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ENGINEERING
AND PROCESS
DEVELOPMENT

Chemical Production of Lactic and Other Acids from Molasses

Reaction Conditions

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The action of lime on molasses, raw sugar, sucrose, glucose, and fructose was investigated with a view to finding the extent to which lactic acid and other acids were produced. Information was also sought on the mechanism of formation of these acids.

The yield of acids was dependent on temperature, time of reaction, proportion of lime to sugar, and sugar molarity. Effects of these variables were interdependent. Yields of 50 to 80% of the theoretical amount of lactic acid were obtained, together with other acids which usually amounted to 25% of the lactic acid. The acids were separated from the nonacidic components as the zinc salts and methyl esters and by extraction with solvents. Pure lactic acid could be isolated by recrystallization of the salts and fractional distillation of the methyl esters but not by solvent extraction alone with the solvents studied. The butyl esters of the mixture of acids were esterified with adipic acid, and the resulting mixture without redistillation was as efficient as dioctyl phthalate for plasticizing vinyl chloride resin. The acidic constituents of the crude lactic acid were investigated qualitatively and quantitatively by chromatographic techniques. The formation of at least 13 acids was demonstrated, lactic acid representing 60 to 80% of the mixture. In connection with recent work on the mechanism of the degradation reactions, the amount of aldehyde and carbon dioxide produced by oxidation of some hydroxy acids with potassium permanganate was determined.

From the standpoint of alkaline degradation, molasses may be regarded as a mixture of sucrose and invert sugar. A study of the reaction mechanism using radioactive sugars must not rely entirely upon ether extraction of the products to isolate pure lactic acid. The alkaline degradation of molasses, although it gives a lower yield of lactic acid than the fermentation procedure, offers possibilities for continuous operation with saving in time and space. The mixture of acidic components may be separated by continuous extraction followed by continuous esterification. Isolation of pure lactic acid requires one further unit operation. The yield of mixed acids is high, and for any application—for example, plasticizer production—which could use the mixture of hydroxy acids, the alkaline degradation procedure would be promising.

CERTAIN carbohydrates undergo degradation in the presence of alkaline reagents. A systematic study of the reactions involved was first made by Nef (34, 35) and extended in more recent years, principally by Evans and coworkers (12, 13). These workers found that the principal product from such a reaction was lactic acid, but other acidic compounds were identified, among them acetic, formic, α,γ -dihydroxybutyric (35), saccharinic (12), and glucic acid (43). The theoretical yield of lactic acid from sugars is 2 equivalents per mole of hexose, and 1 equivalent per mole of pentose or triose. Nef (35) showed that tetroses rearrange in alkaline media to form saccharinic acids almost exclusively. Glucose, fructose, and invert sugar have been converted to lactic acid to the extent of 60 to 67% of the theoretical value (25, 45, 50).

The first extensive work on the formation of lactic acid from sucrose was done by Wolf (4, 54), who used calcium oxide, sodium hydroxide, and barium hydroxide reagents at tempera-

tures of about 200° C. Lactic acid was obtained in yields up to 73% of the theoretical value but was isolated as the pure zinc salt only in 60% yield. Haworth, Gregory, and Wiggins (22) isolated pure zinc lactate from sucrose in yields of 71% of the theoretical amount. More recently the yields of lactic acid from cane sugar and refiner's blackstrap molasses have been investigated by Miyake, Hayashi, and Sano (28), who reported their highest yield as 61.5% from sucrose and 61.3% from molasses, but did not isolate the acid in this yield. Only Braun (6) has patented the production of lactic acid from sucrose and molasses without previous inversion, claiming that the reaction mixture from sucrose contained 72 to 75% of the theoretical yield of acid, which was isolated in 45% yield as calcium lactate and 50% yield as the zinc salt.

Although it has been known for some time that acids other than lactic acid are produced by alkaline degradation of carbohydrate materials, little work has been reported on the isolation of these acids. With a view to finding the extent to which lactic acid and the unknown acids are formed from molasses and raw sugar, the action of calcium oxide and calcium hydroxide on Cuban blackstrap molasses, refiner's blackstrap molasses, beet molasses,

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Table I. Effect of Temperature on Yield of Lactic Acid and Other Acids from Cuban Blackstrap Molasses^a

Expt. No.	Molasses		CaO ^c , Grams	Reaction Temp., ° C.	Reaction Time, Hours	Steam-Volatile Acids, Equiv.	Acid Volatilized			Lactic Acid in Autoclave Liquor		
	Grams	Moles ^b					Continuous esterification, equiv.	Batch Esterification		Equiv.	Equiv. per mole molasses ^b	Theoret. yield, %
								Equiv.	Equiv. per mole molasses ^b			
21	503.5	1.614	130.1	96-107	2.5	0.08	.. ^d	0.56	0.35	0.34	0.21	11
20	507.2	1.626	131.0	134-138	3	0.13	.. ^d	0.82	0.50	0.63	0.39	19
19	504.3	1.618	130.3	168-175	2.5	..	1.44	1.46	0.91	1.23	0.76	38
17	507.2	1.626	131.0	196-201	2.5	0.27	1.60	1.62	1.00	1.33	0.82	41
12	512.0	1.642	132.3	218-222	2.5	0.31	1.61	1.81	1.10	1.47	0.89	45
27	501.8	1.611	129.6	228-236	2.66	0.23	1.63	1.90	1.18	1.55	1.00	50
18	509.5	1.634	131.6	254-260	2.5	0.22	1.91	1.82	1.11	1.51	0.92	46
37	503.1	1.615	130.0	285-295	3 ^d	0.35	0.22	0.20	0.13	6
10	510.5	1.636	131.9	100-102	7	0.15	.. ^d	0.58	0.35	0.38	0.24	12
11	503.7	1.615	130.1	134-139	7.3	0.15	.. ^d	1.00	0.62	0.66	0.41	21
9	516.3	1.656	134.6	156-162	7	..	1.67	1.56	0.94	1.32	0.79	40
4	520.9	1.671	134.6	193-200	7.5	0.33	1.81	1.99	1.19	1.61	0.96	48
2	512.2	1.643	132.3	236-241	7	0.24	1.73	1.92	1.17
36	500.6	1.607	129.3	266-272	7	0.91	0.57	0.55	0.34	17
47	500.3	1.606	202.9	160-168	2.3	1.74	0.99	1.21	0.75	38
46	501.6	1.611	203.5	195-201	2.3	2.10	1.22	1.59	0.98	49
24	500.8	1.608	203.1	228-235	2.67	0.30	1.54	1.94	1.21	1.51	0.94	47
48	500.6	1.607	203.4	253-258	2.5	1.77	1.02	1.28	0.80	40

^a Molasses dissolved in 650 ml. of water, and calcium oxide added to solution.^b Total sugars expressed as moles of invert sugar.^c Molar ratio of 1.436 moles of calcium oxide per mole of invert sugar used in each experiment except 24, 46, 47, and 48, where molar ratio was 2.25.^d Reaction tube blocked.

and raw cane sugar has been investigated. From the results obtained, it is possible to compare the chemical and fermentation procedures for producing lactic acid from these materials.

Procedure

Materials. The molasses and raw cane sugar had the following composition:

	Blackstrap Molasses		Beet Molasses	Raw Cane Sugar
	Cuban	Refiner's		
Sucrose, %	32.90	33.21	47.95	97.79
Invert sugar, %	23.09	19.31	0.35	0.49
Total sugars, mole of hexose per 100 grams of molasses	0.321	0.301	0.297	0.572
Ash (sulfated less 10%), %	8.88	8.10	11.44	0.48
Protein, %	2.69	2.31	6.16	0.28
Fiber, %	0.61	0.24	...	0.06
Chloride, %	1.64	1.32

The calcium oxide and calcium hydroxide were C.P. grade and finely powdered.

Alkaline Degradation Reaction. The reactants were mixed and heated under pressure. The mixtures were agitated by

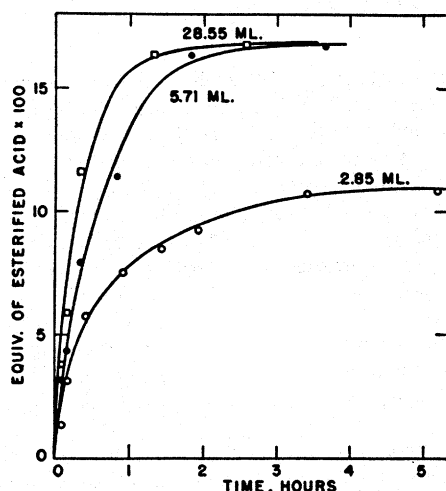


Figure 1. Effect of Catalyst Concentration on Rate of Volatilization of Acids in Crude Lactic Acid

ML. of concd. sulfuric acid per equivalent of acid

rocking the bomb through an angle of approximately 30° at the rate of 58 times a minute. The bomb was electrically heated, and the temperature was controlled and recorded automatically. Unless otherwise stated, the rate of heating was about 4° C. per minute, and the time of reaction was taken from the point when the reaction temperature was reached. The reaction products after removal from the bomb were heated to boiling, and sulfuric acid, suitably diluted, was added until the pH of the solution was 2.0 to 2.2. Owing to the calcium carbonate formed during the reaction, precipitation of calcium sulfate was accompanied by a large evolution of carbon dioxide. The calcium sulfate was filtered, and the precipitate was washed once with about twice its weight of boiling water. The combined filtrates constituted the crude lactic acid, so called because lactic acid was the main component.

Isolation of Acidic Reaction Products. An aliquot of the crude lactic acid solution was evaporated at about 6- to 12-mm. pressure to remove all the water and steam-volatile materials. The aqueous distillate was colorless and contained 0.1 to 0.3 equivalent of acid per mole of molasses (Table I). The acidic constituents were not investigated, but were probably acetic and formic acids, these acids having been identified from similar reactions by previous workers (12, 13). In some cases the aqueous distillate became brown on standing overnight.

The nonvolatile residue from the evaporation was treated batchwise with methanol vapor at 85° to 95° C. in the presence of sulfuric acid catalyst, according to the method described by Filachione and Fisher (14, 15). The methyl esters thus formed were volatilized in the stream of methanol vapor, and the mixture of alcohol, water, and methyl esters was led to a stripping still. Here the major portion of the alcohol was separated and recycled.

During the esterification the methyl esters collected at the base of the stripping column. The esters were colorless until the end of the reaction, when a dark brown material usually came over. Titration and saponification data were obtained on the ester solution to estimate the amount of acid carried over, principally as the ester, by the alcohol vapor.

In view of the fact that the Cuban blackstrap molasses and refiner's blackstrap molasses contained chlorides, which still were present in the crude lactic acid solution, a correction had to be applied for the hydrochloric acid produced by the action of the sulfuric acid catalyst. The hydrochloric acid was estimated by titration with silver nitrate, and the saponification figure was corrected.

The effect of varying the sulfuric acid catalyst concentration was studied, as more than catalytic amounts are required. This series of experiments is described in detail to illustrate the general procedure discussed above.

Five hundred and six grams of Cuban blackstrap molasses (1.622 moles of invert sugar), 130.7 grams of calcium oxide (1.436 moles per mole of hexose), and 650 ml. of water were heated at 195° to 202° C. for 3.5 hours. The resulting mixture was heated to boiling and brought to pH 2.2 with concentrated sulfuric acid (125 ml.) suitably diluted. The precipitated calcium sulfate was filtered and washed, giving a combined filtrate of 2100 ml. A 500-ml. aliquot of this filtrate was evaporated at 6- to 12-mm. pressure in a 500-ml. three-necked flask. When the water had been removed, the aqueous distillate was titrated with standard sodium hydroxide; 0.075 equivalent of acidic material had distilled with the water. To the nonvolatile residue from the evaporation was added 6 ml. of concentrated sulfuric acid. The three-necked flask was attached to a stripping still, and heated on a boiling water bath. When the reaction mixture reached a temperature of 70° to 80° C., methanol, contained in a graduated cylinder, was pumped with a constant-rate bellows pump through a steam-jacketed preheater into the bottom of the crude acid mixture. The vapors were passed to the stripping column, and the esters were collected in suitable fractions during a reaction time of 5.5 hours. These fractions were saponified and titrated to determine the amount of acid volatilized.

Subsequently, 200-ml. aliquots of the crude lactic acid solution were evaporated and esterified as described above, and different amounts of concentrated sulfuric acid catalyst were used. Figure 1 shows the effect of catalyst concentration on the rate of volatilization of acid. Over the range of catalyst concentration investigated, more than 5.7 ml. of concentrated sulfuric acid per equivalent of esterified acid effected complete recovery in less than 5 hours.

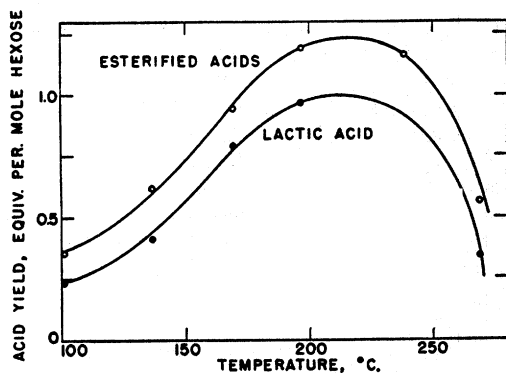


Figure 2. Effect of Temperature on Acid Yield from Cuban Blackstrap Molasses

7-hour reaction time, 2.5 hexose molarity, 1.44 moles of CaO per mole of molasses

Definition of Terms. In view of the fact that the study of this reaction involves five groups of acids, it is pertinent here to define their derivation and meaning.

Crude lactic acid refers to the product from the autoclave reaction which has been acidified to pH 2.0 to 2.2 with sulfuric acid and from which the calcium sulfate has then been separated.

Lactic acid is that fraction of the crude lactic acid indicated by analysis according to the procedure of Friedemann and Graesser (18) slightly modified by C. O. Willits of this laboratory (details to be published later).

Esterified acid is the condensate from the application of the methanol vapor esterification process (14, 15) to the crude acid acid less the hydrochloric acid.

Unknown acids are the esterified acids other than lactic acid.

Steam-volatile acids were obtained when crude lactic acid was evaporated to dryness under reduced pressure at 40° to 70° C.

One mole of molasses is defined as the amount of molasses that contains 1 mole of total sugar, the sugar being expressed completely as hexose.

Effects of Variables on Yields of Lactic Acid and Other Acids

Effects of reaction temperature, time of reaction, proportion of lime to sugar, and molarity of the initial sugar solution on the yields of lactic acid and unknown acids were investigated, with special reference to molasses. The compositions of Cuban and refiner's blackstrap molasses are similar, so that one would expect the two materials to behave similarly under conditions of alkaline degradation. This was found to be the case. Although beet molasses consists principally of sucrose, it behaves more like blackstrap molasses than pure sugar (Table II). This is probably due to the presence of large amounts of protein material which reacts with the sugar.

The investigation of the degradation reaction is summarized in part in Table I, and effects of the variables are shown in Figures 2 to 6. (The data relating to the effect of time of reaction, proportion of lime to sugar, and the molarity of the initial sugar solution on the yields of lactic acid and unknown acids from Cuban blackstrap molasses have been deposited with the American Documentation Institute as also have all the data dealing with the alkaline degradation of refiner's blackstrap molasses.)

Temperature. Figure 2 shows that yields of lactic and esterified acids increased with increase in temperature to a maximum, and then decreased rapidly with further increase in temperature. The yield of esterified acids was greater than that of lactic acid by an amount which, though nearly constant, increased with temperature to an optimum value, which occurred at approximately the same temperature as that of lactic acid. The practical significance of this finding is that the proportion of lactic acid in the esterified acids is greatest at the optimum temperature for lactic acid formation.

The effect of temperature on the yield of acids was modified by the length of the reaction and the proportion of lime to sugar, the optimum temperature being lowered by the increase of these two variables (Table I). The highest yields of acids (1.10 to 1.22 equivalents per mole of molasses) were obtained under the conditions of experiments 2, 4, 12, 18, 24, 27, and 46 in Table I. The highest yields remained approximately the same with these changes in alkali concentration and reaction time.

The yield of lactic acid from molasses at temperatures below approximately 130° C. is thought to result almost entirely from the degradation of the invert sugar already present. Thus, from

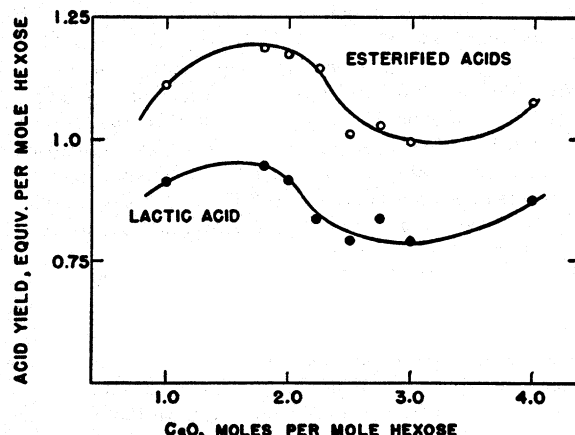


Figure 3. Effect of Proportion of Lime on Yields of Acids from Cuban Blackstrap Molasses

2.5- to 3-hour reaction time, 230° to 240° C. reaction temperature, 2.5 hexose molarity

Table II. Yield of Lactic Acid and Other Acids from Raw Sugar and Beet Molasses

Expt. No.	Materials ^a	Materials ^a		CaO	Moles per mole hexose ^b	Reaction Temp., °C.	Reaction Time, Hours	Acid Volatilized			Lactic Acid in Autoclave Solution		
								Continuous esterification, equiv.	Batch Esterification		Equiv.	Equiv. per mole hexose ^b	Theoretical yield, %
		Grams	Moles ^b	Grams					Equiv.	Equiv. per mole hexose ^b			
1	Raw sugar	400	2.287	147.4	1.12	231-238	2.5	2.60	2.91	1.31	2.01	0.88	44
2	Raw sugar	400	2.287	147.4	1.12	229-238	4.5	..	2.76	1.21	2.27	1.00	50
3	Raw sugar	400	2.287	147.4	1.12	251-260	2.5	2.60	2.82	1.23	2.17	0.95	47
4	Raw sugar	400	2.287	147.4	1.12	234-238	7.5	..	2.63	1.15	2.18	0.95	47
5	Raw sugar	400	2.287	163.7	1.25	234-239	7.5	..	2.51	1.10	2.02	0.88	44
6	Beet molasses	500 ^c	1.485	123.6	1.50	265	2-4 min.	..	1.70	1.15	1.81	1.22	61
7	Beet molasses	500	1.485	200.4	2.25	207-214	2.5	..	1.86	1.26	1.68	1.13	56
8	Beet molasses	500	1.485	200.4	2.25	265-267	0.25	..	1.68	1.12	1.45	1.00	50
9	Beet molasses	67 ^d	0.200	26.7	1.80	255	2-4 min.	..	0.33	1.65	0.28	1.40	70

^a Material dissolved in 1000 ml. of water, and calcium oxide added to solution.

^b Total sugar content expressed as moles of hexose.

^c Beet molasses dissolved in 1500 ml. of water.

^d Beet molasses dissolved in 2000 ml. of water.

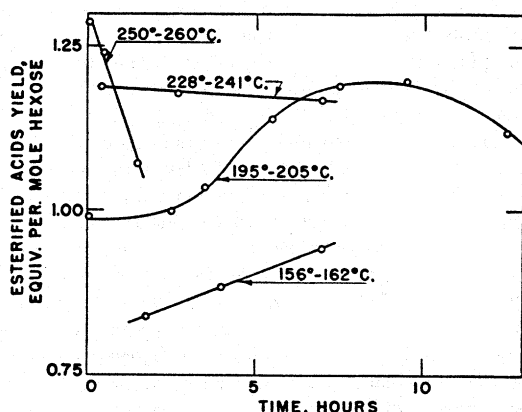


Figure 4. Effect of Reaction Time on Yield of Esterified Acids from Cuban Blackstrap Molasses

1.44 moles of CaO per mole of molasses, 2.5 hexose molarity

Table I, experiments 10, 11, and 9, respectively, the yields of lactic acid based on the invert sugar originally present in the molasses were 0.58 equivalent per mole of invert at 101°, 1.02 at 136°, and 1.99 at 169° C. After 2.5-hour reactions at reaction temperatures similar to those above, the yields were 0.53, 0.97, and 1.90 equivalents of lactic acid per mole of invert sugar, respectively (Table I, experiments 21, 20, and 19). The yields obtained at 101° and 136° were no more than those obtained from glucose, fructose, or invert sugar by other workers, but the high yields at 169° have never been reported before and must therefore include degradation of the sucrose. The yield of lactic acid from sucrose at 100° C. is negligible (5). It is thought therefore that the sucrose does not begin to react completely until between 136° and 160°, which is also suggested by the increasing rate of change of yield with temperature in this range (Figure 2). This assumption is also supported by the fact that sucrose does not invert appreciably in neutral (30) or alkaline (40) media until the temperature is above 130° C., and it is generally accepted that the sucrose must invert before it degrades to lactic acid.

Proportion of Alkali to Sugar. Miyake, Hayashi, and Sano (28) have reported that the yield of lactic acid from refiner's blackstrap molasses increased to a maximum at 6 moles of calcium oxide per mole of molasses, but decreased when the ratio was increased further. Wolf (4, 54), however, found that the yield of lactic acid from sucrose increased until there was a little more than the theoretical amount of calcium oxide present, after which little change in yield was obtained up to a lime-sucrose mole ratio of 3.0. The results obtained in the present investigation indicate that the effect of this variable is complex. The

maximum yields of esterified and lactic acids were obtained from Cuban blackstrap molasses at 1.8 to 2.0 moles of calcium oxide per mole of molasses. A further increase in the proportion of alkali resulted in an 8% reduction in yield at a mole ratio of 2.5 and 3.0, followed by an increase in yield at a mole ratio of 4.0 (Figure 3). The effect did not seem to be dependent on temperature—a similar relationship was observed with refiner's blackstrap molasses at 250° to 260° C. As expected, the same results were obtained with calcium oxide or hydroxide.

Again, changes in this variable did not appreciably change the yield of unknown acid.

There is no logical explanation for this unusual variation of yield with proportion of calcium oxide. Shaffer and Friedemann (45) investigated the effect of alkali concentration by reacting glucose and fructose with potassium hydroxide solutions at 37° to 40° C. They found that the yield of lactic acid reached a maximum in 2 *N* alkali; further increase in the hydroxyl ion concentration produced at first a slight reduction in yield, followed by a smaller increase in yield with 10 *N* hydroxide. These workers, however, always used a large excess of alkali, so that their results may be attributed to the increase in hydroxide ion concentration. It is doubtful whether the changes in yield shown in Figure 3 are due to increase in the hydroxide ion concentration, because in all the experiments the solution presumably was saturated with respect to calcium hydroxide. Solubility studies on calcium hydroxide have not been made at the temperature of the experiments reported here, but it is known that calcium hydroxide has a negative solubility coefficient at lower temperatures.

Time of Reaction. As expected from general kinetic considerations, the time required to obtain the maximum yield of acids depended on the reaction temperature. Thus, at 156° to 162° C. the optimum time was greater than that studied; at 195° to 205° C. the time was 8 hours; at 228° to 241° it was less than 30 minutes; and at 250° to 260° only a few minutes were required (Figures 4 and 5). These results applied to both esterified acids and lactic acid. The amount of esterified acids produced at the optimum times ranged from 1.19 equivalents per mole of molasses at 195° to 205° C. to 1.29 at 250° to 260° C. High reaction temperature and short reaction times are advantageous, therefore, not only for acid yield but also for the chemical degradation of molasses by a continuous operation. A holding time of 8 hours, the optimum reaction time at 200° C., is impracticable with such a heterogeneous reaction mixture, but a holding time of up to 30 minutes is well within the realms of possibility.

The yield of unknown acid ranged from 0.20 to 0.26 equivalent per mole of molasses. The yield of lactic acid, however, varied more widely, indicating that the amount of lactic acid isolated was dependent on time and temperature, in direct contrast to the unknown acids. Figure 5 shows that after the optimum time had been passed the yield of lactic acid decreased at a rate which

was approximately the same between 195° and 240° C. but greatly increased at 250° to 260°. This behavior is probably related to the fact that calcium lactate, when reacted with lime at 230° to 240° C. for 7 hours, is transformed to the extent of 8%, whereas at 250° to 260° C. the figure is 38%.

Sugar Concentration. The effect of sugar concentration on the yield of lactic acid has been extensively studied by Shaffer and Friedemann (45) and Miyake, Hayashi, and Sano (28). The latter group found that the yield of lactic acid from sucrose and refiner's blackstrap molasses reached a maximum as the sugar concentration was decreased. The maximum yield was produced at 25% molasses concentration and 15 to 30% sucrose concentration, the optimum in the case of sucrose depending on the proportion of lime. These results differ from those of Shaffer and Friedemann, who treated glucose with a large excess of calcium hydroxide at 37° to 65° C. and found that the yield of lactic acid continued to increase as the concentration of glucose was reduced. In the alkaline degradation of dihydroxyacetone, the yield of lactic acid approached the theoretical value at very low concentrations (45).

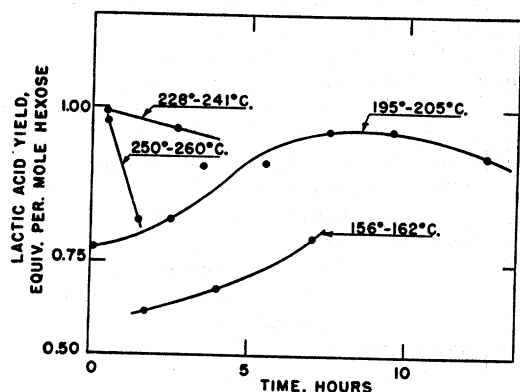


Figure 5. Effect of Reaction Time on Yield of Lactic Acid from Cuban Blackstrap Molasses

1.44 moles of CaO per mole of molasses, 2.5 hexose molarity

The authors' results are in substantial agreement with those of Shaffer and Friedemann. The effect of the initial sugar concentration on the yield of lactic and esterified acids was investigated: The reaction was conducted at 230° to 240° C. for 2.5 hours and 2.5 moles of calcium oxide were used per mole of molasses. The yields of esterified and lactic acids increased as the initial sugar concentration decreased. Similar results were obtained when the amount of alkali was increased to 2.75 moles per mole of molasses. By decreasing the molarity of the molasses in the autoclave charge from 3.0 to 0.1, the yield of lactic acid was increased from 26 to 80% of the theoretical value (Figure 6). The latter appears to be the highest conversion of molasses to lactic acid thus far reported. There is little doubt, however, that had Haworth, Gregory, and Wiggins (22) estimated the lactic acid in their experiments by the oxidation procedure of Friedemann and Graesser instead of by the isolation of double recrystallized zinc lactate, their yields would have been in the region of 80% of the theoretical value.

Continuous Countercurrent Esterification

It has been shown by analysis of crude lactic acid and its resulting esterified acids that the procedure described above for the isolation of the acidic reaction products recovers from 95 to 100% of the lactic acid in the crude solution. In view of this high recovery, the procedure of Filachione and Fisher (14) was investigated. The crude lactic acid solution was concentrated by about fivefold and filtered, concentrated sulfuric acid (13 ml.

per equivalent of lactic acid) was added, and the solution was fed in dropwise at the top of the reaction tower while methanol (8 to 10 ml. per minute) was vaporized into the base. The vapors from the tower were stripped and analyzed as described above for the batch esterification procedure. The yields of acid isolated by this procedure were 80 to 100% of those isolated by the batch procedure (Table I). The concentrated crude lactic acid solution, however, especially that from molasses, had a high solids content. All the solid material accumulated in the reactor until a blockage was produced. The rate of blocking was particularly rapid when the molasses had reacted with lime at temperatures below 140° C. The presence of the salts in the reactor necessitated frequent cleaning, so that the operation, though continuous in principle, was not so in practice.

Plasticizers from Esterified Acids

Several efficient plasticizers for vinyl resins can be prepared from lactic esters (41). One such compound is butyl lactate adipate (42). It was decided to carry out the reactions involved in the preparation of this compound, using the esterified acid isolated from the alkaline degradation reactions.

The esterified acid (1.99 equivalents) was converted to the corresponding *n*-butyl esters by treating with *n*-butyl alcohol (910 ml.) and concentrated sulfuric acid (5.5 grams). The resulting *n*-butyl esters were not distilled but were dissolved in dry ether (550 ml.) and dry pyridine (272 ml.). This solution was cooled in an ice bath, and adipyl chloride (272 grams) was added dropwise with stirring over a period of 2.5 hours. The reaction mixture was allowed to stand overnight and then added to water. The ether solution, after it was washed with water and dried, was distilled, all materials boiling below 90° C. at 0.01-mm. pressure being removed. The residue (286 grams) was dark in color and had a free acidity of 2.9% calculated as acetic acid. A sample of this residue, A, was tested for plasticizer efficiency (test 1291). In view of the high free acidity, a portion of A (73.0 grams) was dissolved in ether, and the solution was washed twice with dilute sodium bicarbonate solution, and then with water, and dried. The ether solution was treated as before, but decolorizing charcoal was present throughout the distillation. The still residue, B, corresponding to A was filtered from the charcoal and found to be lighter in color than A, but still colored. Residue B was tested for plasticizer efficiency (test 1292).

In the vinyl ester plasticizer tests, the resin (Vinylite VYDR) was milled with the plasticizer (35% of the composition) in the presence of lead carbonate stabilizer on a hot mill. It was then molded on a press to produce a sheet 0.08 inch thick. The plasticizer tests are summarized in Table III.

All the plasticizers were compatible. Table III shows that the plasticizers compare favorably with butyl lactate adipate and dioctyl phthalate.

In view of developments on the pyrolysis of lactic acid esters

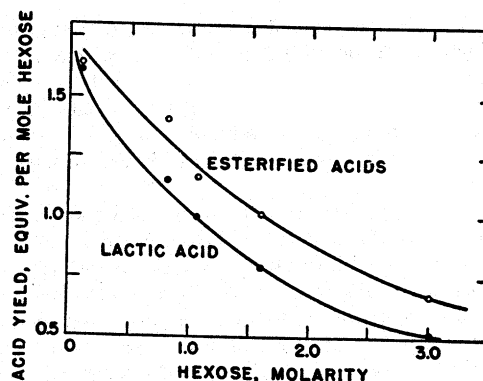


Figure 6. Effect of Sugar Concentration on Yield of Acids from Cuban Blackstrap Molasses

2.5-hour reaction time, 230° to 240° C. reaction temperature.
2.5 moles of CaO per mole of molasses

Table III. Properties of Plasticized Vinylite VYDR

Plasticizer	Tensile Strength, Lb./Sq. Inch	Ultimate Elongation, %	Modulus 100% Elongation, Lb./Sq. Inch	Bend-Brittle Point, ° C.
DOP ^a	3180	395	1270	-35 to -37
BLA ^b	>3140	>380	1070	-30 to -31
1291	>2790	>380	1060	-31 to -32
1292	3140	380	1120	-32 to -34

^a Di-2-ethylhexylphthalate.^b Pure butyl lactate adipate.

to give esters of acrylic acid (8, 47), the process was investigated with the mixture of methyl esters from crude lactic acid. The methyl esters were acetylated and pyrolyzed (47) at 550° to 560° C., borosilicate glass tubing being used for contact material. The yield of methyl acrylate, isolated as a nearly colorless liquid, was 76% of the theoretical. These results indicated that methyl acrylate could be produced from the esterified acids without the isolation of methyl lactate.

Discussion

It is apparent from recent publications that interest in lactic acid, particularly with reference to the plastics field, has increased. Before this interest can be converted to practical application, however, the price of the lactic acid must be appreciably reduced. In any plan to produce relatively low-cost lactic acid, blackstrap molasses must be considered in normal times as one of the most promising raw materials.

Because lactic acid may be prepared in good yield (80 to 95%) by the fermentation process, all lactic acid of commerce has to date been made by fermentation. From an industrial point of view, however, fermentation demands a large amount of space and operational time and maintenance of pure culture. Also, the addition of nutrients adds to the already high solids content in the final crude lactic acid. These disadvantages are absent or at least reduced in the chemical method; the reaction time need be no more than 30 minutes and can be only a few minutes, the reaction is carried out in more concentrated solutions than are possible by fermentation methods, with resulting lower evaporation costs, and a continuous process is feasible.

(Chemical Production of Lactic Acid and Other Acids from Molasses)

Purification Procedures

THE investigation of the chemical production of lactic acid and other acids from molasses was continued with a study of the purification of lactic acid in the crude reaction liquor. The reaction conditions required for a maximum yield of acids are such that a continuous process is feasible, but if the whole production is to be continuous the method of isolation must be equally suitable.

Purification of lactic acid is difficult because of its low vapor pressure, its tendency to undergo self-esterification, and its similarity to water in solubility characteristics. Five types of purification procedures have been reviewed by Smith and Claiborn (46), and knowledge of the subject has been appreciably increased by the more recent work of Fisher and Filachione. Briefly, the methods comprise crystallization of salts, removal or destruction of some impurities by treatment with oxidizing agents, distillation of the free acid or its esters, and solvent extraction. The authors have investigated some of these and other purification

procedures; crude lactic acid obtained by treating Cuban blackstrap molasses with lime was used.

Cuban blackstrap molasses heated at 230° to 240° C. for 30 minutes gave a yield of 1.2 equivalents of esterified acid per mole of molasses. Even assuming a recovery which is 90% of that obtained in the laboratory, by operating on a 7-day week, 24 hour a day, 340 days a year, 5,000,000 pounds of acid could be produced annually with a tubular reactor 10 feet long and 18 inches in diameter, with standard schedule No. 60 steel pipe. For the same annual production of lactic acid by fermentation, Needle and Aries (32) calculated that a total fermentation capacity of 100,000 gallons would be required.

The final crude lactic acid which, depending on reaction conditions, contains 50 to 60% of the theoretical amount of lactic acid and 20 to 25% of this amount of other acids (excluding hydrochloric, formic, and acetic acids), is similar to technical 20% lactic acid of commerce in appearance, and the solids content is no greater than that from the fermentation of Cuban blackstrap molasses. Therefore, the recovery and purification of acids from alkaline degradation are essentially the same as from fermentation liquor. The fermentation method, however, does not concomitantly produce other acids. Pure methyl lactate can be obtained from the esterified acids by fractional distillation, but any development which could use the mixture of acidic products would make the chemical process promising.

A detailed cost analysis would not have any great significance at this stage of the work. Such an analysis for the production of methyl lactate from blackstrap molasses by the fermentation process has been reported by Needle and Aries (32), and apart from the fact that whiting, diammonium hydrogen phosphate, and malt sprouts are used instead of lime, their analysis will probably apply to the chemical process for producing mixed acid esters within the limits of error involved in such an analysis.

It has been stated that anyone considering manufacture of lactic acid should look only to the plastics field. In view of the source of acrylates from petroleum by-products, at this time potential application to the plastics field becomes narrowed principally to alkyd resins and plasticizers. For these uses, there seems no good reason why pure lactic acid should be preferred to lactic acid containing other hydroxy acids. Tests have shown that the mixture prepared from the esterified acids in exactly the way that butyl lactate adipate is prepared from pure lactic acid is as efficient as butyl lactate adipate or dicitol phthalate in its plasticizing effect on vinyl resins.

Recrystallization of the calcium salts (28) and zinc salts (22) has been used recently in isolating lactic acid from alkaline-degraded sucrose solutions. In the authors' hands these methods proved time-consuming or involved appreciable loss of material. At least two recrystallizations were required to isolate the pure lactates from the salts of other organic acids.

Crude lactic acid produced by alkaline degradation of blackstrap molasses is usually dark in color, similar in appearance to the dark-grade, 22% lactic acid of commerce. Its appearance could no doubt be improved by oxidizing some of the impurities, but the chemical purity would be little changed. The color materials are often attributed to a Browning reaction between the sugars and amino acids present in the original sugar juices. Such Browning products are usually polymeric and as such were not expected to dialyze. A preliminary study of dialysis of the

crude lactic acid through cellophane membranes, however, indicated that the color constituents were ionic. Electrodialysis showed some of them to be acidic. Electrodialysis could not be applied to the purification of lactic acid because this acid undergoes electro-oxidation (9, 31, 48).

minutes after the reaction temperature had been attained (70 minutes). After cooling overnight, the reaction product was brought to pH 2.1 with sulfuric acid, and the precipitated calcium sulfate was filtered and washed. The resulting crude lactic acid had a volume of 2045 ml. and contained 1.65 equivalents of lactic acid.

Table IV. Acid Composition of Crude Lactic Acid, Esterified Acids, and Solvent Extracts

Expt. No.	Sample	% Composition of Chromatographic Fractions Having Peak Effluent Volume of						
		30 ml.	80 ml.	150 ml.	250 ml.	380 ml. (lactic acid)	450-end (group B)	30-250 ml. (group A)
1	Crude lactic acid	2.6	0.4	3.6	9.4	67.8	16.3	16.0
2	Esterified acids ^a	1.3	...	4.3	9.7	71.3	13.3	15.3
3	Pentanol extract	2.6	0.4	9.2	13.3	59.0	15.5	25.5
4	Methyl ethyl ketone extract	4.3	0.2	11.7	15.0	53.3	13.6	31.2
5	Benzyl alcohol extract	6.5	0.9	9.0	15.0	58.5	11.1	31.4
6	tert-Amyl alcohol extract	4.7	0.0	6.8	12.2	62.4	8.8	23.7
7	Methyl isobutyl ketone extract	5.1	1.4	14.1	16.9	53.4	8.9	37.5
8	1-Nitropropane extract	33.0	...	20.8	12.1	28.1	5.9	65.9
9	Diethyl ether extract	7.3	...	20.2	22.2	47.0	3.8	52.0
10	Ethyl acetate extract	6.7	0.0	12.8	17.1	56.0	6.2	36.6
11	Second stage water extract of ethyl acetate extraction	0.5	0.2	6.1	14.2	68.7	10.5	21.0

^a Isolated by batchwise esterification of crude lactic acid, as described previously, and subsequent hydrolysis of esters.

One of the most promising methods of recovery is that in which an alcohol vapor is passed through crude lactic acid in the presence of acid catalyst to give the ester of the acids (14, 44, 52). The continuous esterification procedure described by Filachione and Fisher (14) proved to be admirable for a crude lactic acid which contains little solid residue, but high-solids crude material permits only semicontinuous operation because of intermittent blockage of the reactor. Crude lactic acid obtained by the alkaline degradation of molasses had an appreciable solids content. Not only was the ash of the molasses present but some calcium sulfate was produced by the acidulation of the reaction liquor. The calcium sulfate content, which appears to be enhanced by the lactic acid, may be considerably reduced by the concentration and subsequent filtration of the crude lactic acid, but many of the salts from the molasses remain in solution. It would seem that either another method of recovery must be found or an intermediate step introduced to reduce the amount of solids. The problem, however, was complicated by the presence of organic acids, other than lactic, in the crude liquor. For these reasons, a preliminary investigation was made of the acidic constituents of the crude lactic acid.

Analytical Methods

The acids of low volatility in the crude lactic acid were qualitatively analyzed by the paper chromatographic methods developed by Lugg and Overell (26). It was possible to demonstrate the presence of five to nine different acids, the number depending on conditions of the alkaline degradation reaction. However, analysis of the crude lactic acid by paper chromatography gives little indication of the relative amounts of each acid. Minor constituents may not be detected, because an overloading of the paper precludes complete separation. These limitations are overcome to a large extent by the analytical technique of Marvel and Rands (27), who used partition chromatography. In this way it was possible to analyze the crude lactic acid quantitatively and also to estimate the separations, if any, achieved by the methanol vapor esterification process or by extracting the acid with various solvents. The crude acid for this series of experiments was prepared as follows:

A mixture of 500 grams of Cuban blackstrap molasses, 214 grams of calcium hydroxide (1.8 moles per mole of total available hexose), and 650 ml. of water was heated at 255° C. for 2 to 4

Fractionation by Partition Chromatography. A 1-ml. aliquot of the crude lactic acid solution was subjected to freeze drying, and the residual sirup was extracted with 0.4 ml. of *n*-butyl alcohol diluted with 0.6 ml. of chloroform; both solvents were saturated with water. The extract was added to the top of a column prepared from 20 grams of silicic acid and 11 ml. of distilled water according to the directions of Marvel and Rands. The residue was extracted further with 0.1 ml. of *n*-butyl alcohol and 0.9 ml. of chloroform, and this extract was similarly added to the column after the first extract had been forced into the column with a little pressure. The insoluble inorganic residue was further extracted with two 1-ml. portions of chloroform, and the material on the column was developed with the following sequence of solutions:

1. 100 ml. of chloroform
2. 100 ml. of 5% *n*-butyl alcohol, 95% chloroform v./v.
3. 100 ml. of 10% *n*-butyl alcohol, 90% chloroform v./v.
4. 100 ml. of 15% *n*-butyl alcohol, 85% chloroform v./v.
5. 100 ml. of 20% *n*-butyl alcohol, 80% chloroform v./v.
6. 50 ml. of 25% *n*-butyl alcohol, 75% chloroform v./v.
7. 30 ml. of 30% *n*-butyl alcohol, 70% chloroform v./v.
8. 30 ml. of 40% *n*-butyl alcohol, 60% chloroform v./v.
9. 30 ml. of 50% *n*-butyl alcohol, 50% chloroform v./v.
10. 30 ml. of 70% *n*-butyl alcohol, 30% chloroform v./v.
11. 200 ml. of 100% *n*-butyl alcohol

Each solution was saturated with water, and the procedure of solvent addition and effluent titration followed that described by Marvel and Rands. The 10-ml. unit effluent fractions were titrated with 0.02 *N* sodium hydroxide; phenol red indicator was used and enough ethyl alcohol added to maintain a homogeneous system.

The calculated percentage composition of the acidic constituents is summarized in experiment 1 of Table IV. The various acid fractions are identified here by their peak effluent volumes. Lactic acid has a peak effluent volume of 380 ml. Because it was of little interest in this study to determine the amounts of the individual acids eluted from the column after lactic acid, no attempt was made to achieve complete fractionation in this region; their total acid content is given in Table IV under group B.

Solvent Extraction of Crude Lactic Acid

Solvent extraction of crude lactic acid obtained by fermentation methods has been extensively studied and used in commercial

processes (36). Leonard, Peterson, and Johnson (24) determined the distribution coefficient of lactic acid between water and fifty organic solvents and concluded that amyl alcohol and isophorone were most suited to their problem. Amyl alcohols have the disadvantage of removing much of the color and other materials from crude lactic acid. Alternative solvents which have been suggested include ethyl acetate (39), nitroparaffins (49), and ethers (10, 23). Although poor extractants, the ethers, particularly isopropyl ether (36), have been used to produce refined lactic acid.

The crude lactic acid was extracted with several solvents which were chosen either for good extracting properties, commercial availability, or ability to hold the extracted color when re-extracted with water. After equal volumes of the crude lactic acid and solvent had been mixed and had attained equilibrium, samples of the aqueous and solvent layers were analyzed for total acidity and chloride. A sample of the solvent phase was then extracted with an equal volume of water, and the concentration of acid in each layer was determined. The results are summarized in Table V. The solvent phase from the first extraction was also analyzed by partition chromatography exactly as described above for the crude lactic acid. The percentage composition of the acidic constituents is summarized in experiments 3 to 11 of Table IV.

Results

During the development of the flowing partition chromatogram, the developing solvents become more similar to water in solubility characteristics as the fractionation proceeds. The fractionation of acids occurs on a partition column by virtue of the fact that some of the individual acids favor the stationary aqueous phase more than the moving organic phase until the moving phase becomes similar enough to water to favor a good partition. Therefore, the order in which acids from a mixture are eluted from the column indicates the type of fractionation

amyl alcohol—showed the least selectivity for any group of acids. The other extreme was illustrated by 1-nitropropane, which was selective for group A acids.

The distribution coefficients were lower for the mixture of acids than for pure lactic acid. The alcohols and methyl ethyl ketone were the best extractants, followed by ethyl acetate, methyl isobutyl ketone, and diethyl ether in that order. In general, the coefficients for the mixed acids varied in the same order as those for pure lactic acid.

All the solvents extracted a large amount of color from the crude liquor and, except for benzyl alcohol and ethyl acetate, this color was extracted by water in the second extraction stage. Both benzyl alcohol and ethyl acetate have distribution coefficients which would be satisfactory for extracting the acids from the crude liquor and isolation of these acids from the extract with water. Ethyl acetate was also one of the better solvents for extracting lactic acid from the crude liquor at the expense of the group B acids. For these reasons, the water extract of the second stage for this solvent was analyzed. It was found that the composition of the crude lactic acid had been changed such that the group A acids were concentrated at the expense of the group B acids. The color of the aqueous solution was light brown.

The crude lactic acid contained all the chlorides originally present in the molasses. Again, most of these chlorides appeared in the esterified acids as hydrochloric acid, owing to the sulfuric acid catalyst. The hydrochloric acid was undesirable, particularly from corrosion considerations, and for this reason, the distribution coefficients of chloride for the different solvents were studied. In general, these values varied in a similar manner to those for the acids. Ethyl acetate was a good compromise between the two extremes of Pentasol and benzyl alcohol.

Conclusion

It is apparent from the composition of crude lactic acid obtained by the alkaline degradation of molasses that pure lactic

Table V. Extraction of Acids from Crude Lactic Acid

Solvent	K, Lactic Acid ^a	First Stage, Crude Acid to Solvent				Second Stage, Solvent to Water			
		Vol. change in aq. phase, %	Acid		Chloride		Vol. change in aq. phase, %	Acid	
			C _w	K	C _w '	K		C _s	K
Pentasol	2.3	-10	0.78	1.79	0.12	1.61	+9	0.25	1.37
tert-Amyl alcohol	..	-28	0.64	1.14	0.12	3.76	+20	0.24	0.98
Benzyl alcohol	2.3	-12	0.77	1.75	0.12	Very high	+6	0.25	1.36
Methyl isobutyl ketone	7.9	-2	0.98	4.58	0.11	12.17	..	0.15	2.15
Methyl ethyl ketone	..	+28	0.63	1.19	0.08	4.29	One layer
Diethyl ether	9.8	+6	1.00	5.87	+11	0.12	2.45
Ethyl acetate	4.1	+2	0.91	2.96	0.06	6.39	+12	0.21	1.80

^a Leonard *et al.* (24).

C_w = concn. of acid in aq. phase, meq. per ml.

C_s = concn. of acid in solvent phase, meq. per ml.

C_w' = concn. of chloride in aq. phase, meq. per ml.

$$K = \frac{C_w}{C_s}$$

which one could obtain by the solvent extraction of an aqueous solution of the acid mixture. In general, one would expect a concentration of the acids with lower peak effluent volumes, the degree of concentration depending on the similarity of the extractant to water. This was found to be the case with the acids in crude lactic acid.

Taking lactic acid as the point of reference in the fractionation, the group of acids eluted before it, group A, were found to be approximately equal in amount to those of group B. It follows, therefore, that the extraction of crude lactic acid will result in a concentration of the group A acids at the expense of group B and also possibly lactic acid. The solvents most similar to water in solubility characteristics—for example, Pentasol (synthetic

acid cannot be easily isolated by solvent extraction alone, unless a solvent of unusual properties is found. The pure acid can be obtained, however, by fractional distillation of the mixture of esterified acids obtained by applying the alcohol vapor process to the crude liquor. This esterification process also increases the proportion of lactic acid in the mixture. Because the problems in applying the esterification process continuously are principally due to inorganic materials in the crude liquor, which can be practically eliminated by solvent extraction, extraction followed by esterification constitutes a feasible continuous process for purification, particularly if the two unit operations involve the same alcohol. Such a process using *n*-butyl alcohol would probably result in a highly desirable *n*-butyl lactate, but time did not permit verification of this postulate.

Observations on Alkaline Degradation of Sugars

REX MONTGOMERY

IN VIEW of current interest in problems involving the alkaline degradation of sugars, as indicated by recent publications (2, 20, 21), the effects of alkaline reagents on sucrose, glucose, and fructose were studied further.

Effects of Variables on Yields of Lactic Acid and Other Acids. Sucrose, glucose, and fructose were treated with an aqueous solution of lime at temperatures between 94° and 270° by the procedure described above for molasses. Table VI gives the reaction conditions and yields of lactic acid, estimated by a slight modification of the method of Friedemann (18, 19). Also included in Table VI are the yields of acids which were isolated by esterification of the reaction products by the methanol vapor process of Filachione and Fisher (14, 15), and which consist of lactic acid and all other acids that form volatile methyl esters. The effects of reaction conditions on the yield of acids from sucrose, glucose, and fructose were similar to those observed for molasses. In the extensive study with molasses, it was found that the effects of temperature, time of reaction, proportion of lime to sugar, and initial sugar concentration were interdependent. This was also evident in the more limited range of experiments on the pure sugars. The highest yield of acids was obtained by heating a 0.1 M solution of sucrose with lime to 250°

to 260° and maintaining this reaction temperature for a few minutes. Little advantage appeared to be gained by using a large excess of lime. The yield of acid from sucrose was greater than that from glucose or fructose. This was expected, since sucrose has a furanoside structure, which produces more lactic acid than a pyranoside (54). Table VI shows that in all cases the yield of esterified acids was greater than that for lactic acid. The two values varied by 0.1 to 0.3 equivalent of acid per mole of hexose, the difference representing the amount of acids other than lactic separated by the esterification process.

At temperatures above 230° to 240°, yields of acids from the alkaline degradation of molasses decreased when the optimum reaction time was exceeded. The rate at which the yields decreased became more rapid as the reaction temperature was increased. These observations suggested that some of the acids were decomposing with the formation of carbon dioxide and low boiling acids, which would not be accounted for by the analytical methods applied in this study. For these reasons, the effect of lime on calcium lactate was studied at temperatures between 230° and 370°. At all temperatures, there was formation of carbon dioxide and loss of lactic acid, the amounts becoming appreciable when the reactants were held at 254° to 260° for 7

Table VI. Yield of Lactic Acid and Other Acids from Alkaline Degradation Reactions

Expt. No.	Carbohydrate, Init. Concn., Moles/Liter	CaO, Moles/Mole Carbohydrate ^a	Reaction Time, Hours	Reaction Temp., ° C.	Steam-Volatile Acids, Equiv./Mole Carbohydrate ^a	Acids from Batch Esterification, Equiv./Mole Carbohydrate ^a	Lactic Acid, Equiv./Mole Carbohydrate ^a
Sucrose							
1	0.59	2.25 ^b	2.5	131-142	..	0.96	0.92
2	0.59	2.25 ^b	2.5	156-164	0.16	2.57	2.19
3	0.59	2.25 ^b	2.67	199-208	0.17	2.61	2.26
4	1.17	2.25	2.3	188-195	..	2.49	2.06
5	1.17	2.25	2.5	234-238	..	2.63	2.16
6	1.17	2.25	7.25	232-237	..	2.35	1.89
7	1.17	2.25	5.5	233-238	..	2.49	2.16
8	1.17	2.50	2.75	251-258	..	2.23	1.68
9	1.17	2.96	7.3	229-236	..	2.05	..
10	1.17	2.96	7	232-237 ^b	..	1.93	1.68
11	1.17	3.60	2.5	230-238	..	2.23	..
12	0.10	3.60	2-4 min.	257	0.24	2.89	2.67
Glucose							
13	1.17	1.25	2.67	94-96	0.11	0.42	0.29
14	0.20	1.25	6.83	131-134	0.21	0.65	0.40
15	1.17	1.25	2.75	130-144	0.13	0.48	0.33
16	1.17	1.25	2.5	158-175	0.17	0.61	0.40
17	0.28	2.00 ^c	7	195-198	..	0.89	0.61
Fructose							
18	1.11	1.25	2.5	95-96	0.10	0.56	0.35
19	1.17	1.25	3	130-140	0.17	0.60	0.32
20	1.17	1.25	2.25	168-173	0.18	0.58	0.47
21	0.28	1.00	7.5	194-199	..	0.86	0.77
Calcium Lactate Pentahydrate							
22	0.65	1.0	0.5	230-240	0.09	1.84	1.84
23	0.65	1.0	2.75	233-240	0.04	1.90	1.88
24	0.65	1.0	5	231-238	0.14	1.78	1.78
25	0.65	1.0	7	230-237	..	2.10	1.84
26	0.65	1.0	2-4 min.	258	0.05	1.90	1.78
27	0.65	1.0	1.25	254-260	0.03	2.44	1.84
28	0.65	1.0	7	255-260	..	1.62	1.24
29	0.65	1.0	2-4 min.	360-370	0.23	1.20	1.00
Cuban Blackstrap Molasses							
30	1.63	1.44	3	134-138	0.08	0.50	0.39
31	1.64	1.44	2.5	218-222	0.19	1.10	0.89
32	1.61	1.44	2-4 min.	253-254	0.15	1.20	0.97
33	1.61	1.44	2.67	228-236	0.14	1.18	0.97
34	1.63	1.44	2.5	196-201	0.17	1.00	0.82

^a One mole of molasses is defined as amount of molasses which contains 1 mole of sugar, sugar being expressed completely as hexose.

^b Reaction temperature attained in twice usual time.

^c Calcium hydroxide.

Table VII. Paper Chromatography of Acids from Alkaline Degradation Reactions

Spot No.	Chromatograms, R_f						Synthetic mixture ^b
	Sucrose 4 ^a	Sucrose 5 ^a	Glucose 16 ^a	Fructose 20 ^a	Calcium lactate 25 ^a	Molasses 33 ^a	
1			0.04		0.04	0.04	
2	0.21		0.22	0.21		0.19	
3	0.28	0.25				0.28	0.27
4			0.31	0.32			
5	0.41	0.38	0.40	0.41		0.40	0.41
6			0.50	0.51		0.50	
7	0.64	0.61	0.62	0.63	0.62	0.62	0.62
8	0.74	0.72	0.74	0.75	0.73	0.73	
9	0.82	0.81	0.84	0.84		0.81	

^a Experiment numbers same as Table VI.^b Mixture consisted of lactic, glycolic, α,β - and α,γ -dihydroxybutyric acids.

hours or 360° to 370° for a few minutes (Table VI). The esters of acids other than lactic were also isolated with methyl lactate from the esterification of the reaction products. The amount of unknown esters increased with higher reaction temperature and longer time.

Analysis of Reaction Products

It has been shown in this study and also by previous workers (12, 16, 33-35, 53) that acids other than lactic are produced by the alkaline degradation of sugars. The acidic products of low volatility from sucrose, glucose, and fructose were therefore submitted to qualitative analysis by a paper chromatographic technique (7) and to quantitative analysis by columnar partition chromatography (27).

Qualitative Analysis. Nef has estimated that 116 products are theoretically possible from the reaction of glucose with strong alkaline reagents. Among these products, formic (12), acetic (12), oxalic (16), α,γ -dihydroxybutyric (33-35, 51), and glucic acids (53) and the various saccharinic acids (12) have been identified, in addition to lactic acid, from alkaline degradation reactions in which oxidizing agents—for example, hydrogen peroxide—were absent. Indications of the formation of glycolic acid have also been reported (16). Of these acids, formic and acetic are too volatile to be detected by paper chromatography and glucic acid is not stable at the temperature of the alkaline degradation reactions investigated (37). Chromatography of the remaining acids showed that oxalic acid has an R_f value of 0.05; the corresponding values for α,γ -dihydroxybutyric, glycolic, and lactic acids are 0.27, 0.41, and 0.62, respectively (7). The saccharinic acids were not investigated, but from comparisons with hexuronic and hexonic acids (7) would be expected to have R_f values of less than 0.05.

The qualitative analysis by paper chromatography of the acid mixtures resulting from the alkaline degradation reactions was similar to that described by Lugg and Overell (26). The chromatograms were developed using the organic phase of an equilibrated mixture of equal parts of 1-pentanol and 5 *M* aqueous formic acid. The positions of the acids were located by spraying with bromophenol blue (0.04%) adjusted to slight alkalinity. Additional evidence of the nature of the acids was obtained by spraying separate chromatograms with solutions of ammoniacal silver nitrate and ammonium vanadate in water and ceric ammonium nitrate in 1 *N* nitric acid, according to methods described by Buch, Montgomery, and Porter (7). The results are summarized in Table VII.

All the acids reduced ceric ammonium nitrate, which was considered good evidence that they contained hydroxyl groups. Any other type of oxidizable grouping would probably not be stable under the alkaline degradation reaction conditions. The acids also reacted with ammonium vanadate and ammoniacal silver nitrate, but these reagents gave little information concerning their general structure.

Spot 7 was identified as lactic acid. Spot 3 corresponded in R_f value to α,γ -dihydroxybutyric acid (7), but unlike the unknown, the pure acid produced a brilliant yellow spot on paper when sprayed with ammonium vanadate solution. α,γ -Dihydroxybutyric acid is known to lactonize in acid solution and therefore would be present mainly as α -hydroxybutyrolactone, which could not be detected by the methods employed.

Glycolic acid and spot 5 had similar R_f values and reacted in an identical way with the spray reagents. The high concentration of acid decolorized ceric ammonium nitrate; ammonium vanadate produced a yellow color immediately; and the ammoniacal silver nitrate spray gave a yellow fluorescence under ultraviolet light 4 hours after the chromatogram had been sprayed (7). It was thought that spot 5 might be a mixture of glycolic and α,β -dihydroxybutyric acids; the latter has an R_f value of 0.36. Under normal chromatographic conditions, these two acids cannot be separated, and the reactions of the two acids with the spray reagents used in this investigation were similar. However, by allowing the descending chromatogram to run for 60 hours, the two acids were separated, and it was found that spot 5 most frequently behaved like glycolic acid but occasionally a faint spot was detected which corresponded to α,β -dihydroxybutyric acid.

Spots 8 and 9 were similar in R_f value to lactylactic acid, R_f 0.74, and lactyllactyllactic acid, R_f 0.82 (7), respectively. However, the amounts of these acids are small in solutions of about 10% lactic acid concentration, such as were produced by the alkaline degradation reactions described above (29). It was thought, therefore, that these polymers may have been produced in the mixture while the aqueous aliquot was drying on the paper prior to development. However, the paper chromatography of a mixture of lactic and sulfuric acid gave no evidence of lactic acid polymers. Moreover, fourfold dilution of the unknown solution followed by refluxing for 24 hours, conditions known to hydrolyze any lactic acid polymers, did not remove the acids in question from the chromatogram. It was concluded that the acids in spots 8 and 9 were produced during the degradation reaction and were not lactic acid polymers.

Quantitative Analysis. Aliquots of the acid solutions containing 10 to 20 meq. of acid were evaporated at room temperature under about 1-mm. pressure, and the residues were quantitatively analyzed by the partition chromatographic procedure of Marvel and Rands (27); a column prepared from 20 grams of silicic acid and 11 ml. of water was used. The acid not eluted from the column by this procedure was estimated by stirring the silicic acid with water for several hours, followed by filtration and titration of the aqueous solution with 0.02 *N* sodium hydroxide.

It became apparent that the order in which the acids were eluted from the silicic acid-water columns with chloroform-*n*-butyl alcohol solvents was the same as that in which they were developed in the paper chromatographic analysis. Owing to the limitations of paper chromatography discussed above, more acids were shown to be present by columnar partition chromatography. The proportions of the acids are summarized in Table VIII, in which the acids are characterized by their peak effluent volumes. Lactic acid, peak effluent volume 380 ml., accounted for 60 to 80% of the total acid isolated from the column. The mixture of acids from sucrose contained a greater proportion of lactic acid than that from the hexoses or molasses. The next most important acid was that at peak effluent volume 240 ml., which corresponded to the acid of R_f value 0.74. The acids with peak effluent volumes of 590, 630, and 730 ml. were not detected as separate spots on the chromatogram, but in most samples analyzed quantitatively two of these three peaks were obvious in the chromatograph. Pure glycolic and α,β -dihydroxybutyric acids had peak effluent volumes of 590 and 740 ml., respectively. There was some evidence, therefore, of the formation of β -lactones in alkaline degradation reactions. Little correlation could be found between the proportions of the acids and the reaction conditions for their formation.

Table VIII. Quantitative Composition of Acid Mixture from Alkaline Degradation Reactions

Expt. No. ^a	Peak Effluent Volume, Ml.												Not eluted ^b
	30	80	150	240	380	520	590	630	730	850	960	1050	
	Composition of Chromatographic Fractions, % of Total Acid												
Sucrose													
1	0.4	0.3	1.2	2.8	65.2	0.5	2.5	...	2.1	2.4	0.2	3.1	7.0
4	1.1	0.4	2.4	8.5	77.6	0.5	4.0	2.0	1.3	1.2	...	0.8	0.9
5	1.4	0.3	3.3	6.5	78.8	6.8	1.0	0.7	0.5	0.9	0.5
6	0.9	0.2	3.1	7.5	80.7	...	4.1	1.4	0.1	1.1	0.9
8	1.7	0.4	3.4	10.0	75.4	3.3	1.6	0.9	1.2	1.3	0.8
12	2.3	0.0	4.2	9.2	80.1	1.8	...	0.1	2.1
Glucose													
A ^c	0.3	...	0.3	2.0	73.6	1.2	2.9	...	4.8	3.6	1.6	3.3	6.5
Fructose													
21	3.7	0.0	3.1	9.5	58.6	0.9	5.7	3.8	2.9	1.1	1.0	1.3	8.4
B ^c	0.4	0.0	0.8	6.0	61.4	1.6	...	3.8	4.9	3.3	2.7	8.4	6.7
Calcium Lactate													
25	1.0 ^d	2.1	96.2	0.8
27	...	0.5	1.2 ^d	3.0	89.5	5.8
Molasses													
30	1.1	0.0	3.0	9.3	62.6	...	2.4	2.4	3.4	6.2	0.5	6.4	3.3
31	1.7	0.4	4.7	9.3	65.6	2.5	5.8	...	2.3	...	1.4	3.2	3.2
32	2.0	0.5	3.7	7.1	64.6	2.0	4.2	4.5	2.5	1.3	1.2	0.6	5.6

^a From Table VI.

^b Acids eluted from column by chloroform-*n*-butyl alcohol solvents were estimated by stirring silicic acid with water for several hours followed by filtration and titration of aqueous extract with 0.02 *N* sodium hydroxide.

^c Hexoses (0.4 gram) heated for 40 hours at 35–50° C. with 13 ml. of 0.52 *N* barium hydroxide.

^d Peak effluent volume 170 ml.

Although on a silicic acid column the acids produced from the reaction of calcium lactate and lime behaved like the acid of spot 8 on the paper chromatogram and acids of peak effluent volume 80, 150, and 240 ml., the amounts of the acids from calcium lactate were too small to account for the corresponding acids from the alkaline degradation of the sugars (Table VIII). Additional reactions must therefore be involved.

Reaction Mechanism

The analysis by chromatographic techniques of the products from the degradation of sucrose, fructose, and glucose has demonstrated the formation of at least thirteen different acids. The mode of formation of acids, particularly lactic acid, was studied by Nef (33–35) and Evans (12). More recently, investigations on the problem have been reported by Bamford *et al.* (2) and Gibbs (20). Gibbs tested the mechanism of lactic acid formation suggested by Evans with the aid of C¹⁴-labeled glucose. Glucose-1-C¹⁴ and glucose-3,4-C¹⁴ were degraded with potassium hydroxide, and an ether extract of the acidic products was subjected to degradation to determine the amount of radioactivity in the different carbon atoms. It was assumed, however, that lactic acid was the only acid in the ether extract which would be degraded. Previous workers have shown that glycolic acid (16), α -hydroxybutyrolactone (51), and other unknown acids are extracted by ether and may therefore undergo oxidation by permanganate in a manner similar to that of lactic acid. This possibility was investigated by the determination of aldehyde and carbon dioxide formed from some hydroxy acids when subjected to the oxidation procedure employed by Gibbs.

Three gas absorbers (designed by C. O. Willits of this laboratory; details to be published later) were connected in series. The first was charged with sodium bisulfite to remove any aldehyde. This absorber was followed by one containing 0.1 *N* potassium permanganate in 0.1 *N* sulfuric acid to remove any sulfur dioxide from the bisulfite but to permit carbon dioxide to pass to the final absorber, where the carbon dioxide was trapped in 0.1 *N* sodium hydroxide. The first gas absorber was connected to the top of the reflux condenser of the reaction flask (11). Carbon dioxide-free air was drawn through the apparatus during the reaction. The conditions for oxidation of the acids studied were those of Friedemann (18, 19), except that a slightly higher concentration of potassium permanganate was used. The aldehyde produced by the reaction was estimated, again by the

method of Friedemann, and the carbon dioxide was determined by titrating the alkali from the trap with 0.05 *N* hydrochloric acid to phenolphthalein and methyl red end points in the usual way. A blank was made for every other estimation. The results for the oxidation of glycolic acid, diglycolic acid, α -hydroxybutyrolactone, α,β -dihydroxybutyric acid, calcium glycerate, and lactic acid are given in Table IX.

Glycolic, glyceric, and α,β -dihydroxybutyric acids gave high yields of carbon dioxide, and glyceric and α,β -dihydroxybutyric acids also gave appreciable yields of aldehyde. α -Hydroxybutyrolactone and diglycolic and α,γ -dihydroxybutyric acids produced smaller amounts of carbon dioxide and aldehyde. Of many acids studied previously (17), only lactic acid gave the theoretical amount of aldehyde. It is possible that the unknown acids in the ether extract may also oxidize in a manner similar to those reported above. These facts should therefore be taken into consideration when studying the alkaline degradation of carbon-labeled sugars.

A kinetic analysis of the alkaline degradation of glucose and fructose was made by Bamford *et al.* (2), who reported that all the reactions are of first order with respect to the sugar concentration. In their study, however, it was assumed that all the acids except lactic form lactones when heated to 100° at a pH of about 1.5. This was not confirmed by the chromatographic analysis of the solutions of acidic products before and after being heated to boiling, and as suspected by Bamford, the reaction constants were therefore not absolute for lactic acid.

Conclusion

The mechanism proposed by Bamford *et al.* for the formation of lactic acid is not consistent with the work of Evans or Gibbs. Probably the most fitting mechanism so far proposed is that of reverse aldol condensation (33). Following the mechanism for aldol condensations suggested by Alexander (1) and bearing in mind that the reducing sugars may form several enediol states, most of the observations on the alkaline degradation of sugars can be brought into line.

Table IX. Aldehyde and Carbon Dioxide Formed by Oxidation of Acids with Acidified Potassium Permanganate

Acid	Moles $\times 10^{-4}$	Moles CO ₂ per Mole Acid	Mole Aldehyde per Mole Acid
Glycolic	0.604	1.05	0.00
	0.417	1.04	...
Diglycolic	0.198	0.20	0.00
	1.293	0.13	...
α -Hydroxybutyrolactone	1.417	0.05	0.03
α,γ -Dihydroxybutyric (sodium salt)	1.847	0.10	0.06
Glyceric (calcium salt)	0.111	2.26	0.56
α,β -Dihydroxybutyric	0.282	1.00	0.57
	0.243	1.38	0.61
Lactic	0.199	1.00	...
	0.199	1.00	...

It is seen that the chromatographic analysis of the acidic constituents from the alkaline degradation of sucrose and its constituent sugars lends support to the view that sucrose first reacts by cleavage of one of the glycosidic bonds such as is found in the methanolysis of sucrose to form glucose and α -methyl- α -fructofuranoside (3). However, the high yield of lactic acid from sucrose is not consistent with the actual formation of glucose and fructose. Rather, it would seem that intermediates are formed, probably ionic forms of these sugars, which could give glucose and fructose, but under the reaction conditions produce lactic

and other organic acids. The chromatographic behavior and proportion of these acids from glucose, fructose, and sucrose are evidence for the chemical similarity of the sucrose intermediates with glucose and fructose. Their difference lies in the fact that whereas the sucrose intermediates undergo degradation principally to lactic acid, glucose and fructose react concomitantly in other ways which exclude 50 to 70% of these sugars from lactic acid formation. Thus, the reaction solution from these hexoses is very dark, while that from sucrose is light in color. Support for the rapid reaction of the sucrose intermediates is found in Table X, in which it is seen that although sucrose reacts incompletely after 2.67 hours at 131–135° C. no reducing sugars are present.

Table X. Effect of Temperature on Degradation of Sucrose with Lime

Expt. No.	Reaction Temp., ° C.	Sugars in Reaction Product, Grams	
		Reducing sugars ^b	Sucrose
1	95	1.0	186.9
2	131–135	0.0	122.3
3	162–171	0.0	0.0

^a In each experiment, 200 grams of sucrose in 1000 ml. of water treated with 97.3 grams of calcium hydroxide for 2.67 hours.

^b Expressed as invert sugar.

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Literature Cited

- (1) Alexander, E. R., "Principles of Ionic Organic Reactions," p. 176, New York, John Wiley & Sons, 1950.
- (2) Bamford, C. H., Bamford, D., and Collins, J. R., *Proc. Roy. Soc., A* 204, 85 (1950).
- (3) Berner, F., *Ber.*, 66B, 1076 (1933).
- (4) Bernhauer, K., and Wolf, H., *Biochem. Z.*, 219, 232 (1930).
- (5) Beythien, K., Parcus, E., and Tollens, B., *Ann.*, 255, 222 (1890).
- (6) Braun, G. (to Standard Brands, Inc.), U. S. Patent 2,024,565 (1935); Brit. Patent 400,413 (1933).
- (7) Buch, M. L., Montgomery, R., and Porter, W. L., *Anal. Chem.*, 24, 489–91 (1952).
- (8) Burns, R., Jones, D. T., and Ritchie, P. D., *J. Chem. Soc.*, 1935, 400–6.
- (9) Carpenisceanu, G., *Compt. rend.*, 198, 460 (1934).
- (10) Dietzel, R., and Schmitt, P., *Z. Untersuch. Lebensm.*, 63, 369 (1932).
- (11) Edwards, H. T., *J. Biol. Chem.*, 125, 571 (1938).
- (12) Evans, W. L., *Chem. Revs.*, 31, 537 (1942).
- (13) Evans, W. L., Edgar, R. H., and Hoff, G. P., *J. Am. Chem. Soc.*, 48, 2665 (1926).
- (14) Filachione, E. M., and Fisher, C. H., *IND. ENG. CHEM.*, 38, 228 (1946).
- (15) Filachione, E. M., Lengel, J. H., and Fisher, C. H., *Ibid.*, 37, 388 (1945).
- (16) Fischler, F., Taufel, K., and Souci, S. W., *Biochem. Z.*, 208, (1929).
- (17) Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 73, 335 (1927).
- (18) Friedemann, T. E., and Graeser, J. B., *Ibid.*, 100, 291 (1933).
- (19) Friedemann, T. E., and Kendall, A. I., *Ibid.*, 82, 23 (1929).
- (20) Gibbs, M., *J. Am. Chem. Soc.*, 72, 3964 (1950).
- (21) Haskins, J. F., and Hogsed, M. J., *J. Org. Chem.*, 15, 1264 (1950).
- (22) Haworth, W. N., Gregory, H., and Wiggins, L. F., *J. Soc. Chem. Ind.*, 65, 95 (1946).
- (23) Klapproth, W., Brit. Patent 280,969 (Nov. 22, 1926).
- (24) Leonard, R. H., Peterson, W. H., and Johnson, M. J., *IND. ENG. CHEM.*, 40, 57 (1948).
- (25) Lock, R. H., U. S. Patent 2,382,889 (1945).
- (26) Lugg, J. W. H., and Overell, B. T., *Australian J. Sci. Research, Ser. A*, 1, 98 (1948).
- (27) Marvel, C. S., and Rands, R. D., Jr., *J. Am. Chem. Soc.*, 72, 2642 (1950).
- (28) Miyake, S., Hayashi, K., and Sano, Y., *J. Soc. Trop. Agr. Taihoku Imp. Univ.*, 14, 291 (1942).
- (29) Montgomery, R., *J. Am. Chem. Soc.*, 74, 1466 (1952).
- (30) Montgomery, R., and Wiggins, L. F., *J. Soc. Chem. Ind.*, 66, 31 (1947).
- (31) Muller, F., and Schwab, H., *Z. Elektrochem.*, 33, 568 (1927).
- (32) Needle, H. C., and Aries, R. S., *Sugar*, 44, 32 (1949).
- (33) Nef, J. U., *Ann.*, 335, 191 (1904); 403, 204 (1914).
- (34) *Ibid.*, 357, 301 (1907).
- (35) *Ibid.*, 376, 1 (1910).
- (36) Peckham, G. T., Jr., *Chem. Eng. News*, 22, 440 (March 25, 1944).
- (37) Pelogot, *Compt. rend.*, 5, 26 (1837).
- (38) Pigman, W. W., and Goepf, R. M., Jr., "Chemistry of the Carbohydrates," p. 78, New York, Academic Press, 1948.
- (39) Pont, E. G., Australian Scientific Liaison Office, Washington, D. C., *Australian Tech. Paper* 790.
- (40) Rao, K. A. N., and Gupta, G. N., *Proc. Sug. Technol. Assoc. India, 8th Convention*, 289 (1940).
- (41) Rehberg, C. E., and Dixon, M. B., *J. Am. Chem. Soc.*, 72, 5757 (1950).
- (42) Rehberg, C. E., Dixon, M. B., and Fisher, C. H., *IND. ENG. CHEM.*, 42, 1409 (1950).
- (43) Sattler, L., and Zerban, F. W., *Sugar*, 42, No. 12, 26–7 (1947).
- (44) Schopmeyer, H. H., and Arnold, C. R., U. S. Patent 2,350,370 (June 6, 1944).
- (45) Shaffer, P. A., and Friedemann, T. E., *J. Biol. Chem.*, 86, 345 (1930).
- (46) Smith, L. T., and Claborn, H. V., *IND. ENG. CHEM., NEWS ED.*, 17, 641 (1939).
- (47) Smith, L. T., Fisher, C. H., Ratchford, W. P., and Fein, M. L., *IND. ENG. CHEM.*, 34, 473 (1942).
- (48) Smull, J. G., and Subkow, P., *Chem. Met. Eng.*, 28, 347 (1923).
- (49) Tindall, J. B., U. S. Patent 2,223,797 (Dec. 3, 1940).
- (50) Torstensen, H. H., doctoral dissertation, Ohio State University, 1939.
- (51) Upson, F. W., *Am. Chem. J.*, 45, 458 (1911).
- (52) Wenker, H., U. S. Patent 2,334,524 (Nov. 16, 1943).
- (53) Winter, H., *Z. Ver. Deut. Zuckerind.*, 44, 1049 (1894).
- (54) Wolf, H., *Biochem. Z.*, 210, 458 (1929); 219, 232 (1930).